Modified membrane for medical purposes

Description:

The invention relates to a membrane for medical purposes, modified by treatment with a low-pressure plasma, which consequently has reduced complement activation and thrombogenity in relation to the unmodified membrane.

Membranes for medical purposes have been known for a longer period in the form of flat membranes, hose membranes or hollow fibre membranes, with very high demands with regard to their so-called "biocompatibility" being placed on these membranes (e.g. those for dialysis, haemodialysis, haemofiltration, oxygenation and others) so that the blood flowing past the membranes is adversely affected as little as possible.

A wide range of different properties and parameters are grouped together under the term biocompatibility that essentially determine and influence the blood tolerability of dialysis machines, membranes and other components.

For the definition of blood tolerability, the so-called zero definition has universally gained acceptance in the field of medical treatment of dialysis patients, i.e. the components of such a system and the system itself should fulfil as many of the following requirements as possible:

- no thrombogenic, toxic, allergic or inflammatory reactions
- no destruction of biological cells
- no modifications in plasma proteins and enzymes
- no carcinogenic effects
- no modifications in the surrounding tissue

Therefore, the biocompatibility data of the individual system components, such as for example the degree and nature of sterilisation (asepsis), the design of the housing, the embedding material of the actual membrane and some other factors decisively determine the biocompatibility of the whole system. Within the context of the present invention, the biocompatibility of the membrane material and the membrane itself will be assessed above all. Essential aspects of biocompatibility include for example thrombogenity, complement activation, leukopenia, in addition to the filtering coefficients for β2 microglobulin, albumin and other substances.

A series of methods have been suggested in order to influence the characteristics of such a membrane with regard to improvement of biocompatibility. The membrane material may therefore be chemically modified for example, in order to obtain a more active or less active membrane surface which subsequently has an effect on specific factors among those mentioned above.

Chemically modified membranes, above all dialysis membranes, that show improved biocompatibility are described for example in the DE-OS 39 01 945, DE-OS 39 01 946, DE-OS 39 01 947, DE-OS 38 14 326, DE-OS 35 24 596, DE-PS 33 41 113 and EP-A-0 459.

These membranes admittedly demonstrate during dialysis and other applications too a marked reduction in C5a complement activation in comparison to unmodified membranes, but partly show however in contrast to conventional dialysis membranes, such as Cuprophan® for example, an increase in thrombogenity and problems may occur when treating patients.

In general however, the procedures for chemically modifying membranes have proved complicated and uneconomical. Furthermore, it often occurs that only one of the parameters that influence biocompatibility is improved, whereas another or several others - particularly thrombogenity - are made worse.

It has therefore also been suggested already that the membrane material be influenced by physical effect, by treatment with a plasma for example. Plasma in this case in the sense of the invention means a gas in the plasma state (after LANGUMUIR). A highly ionised gas with special properties is involved which are based on the interactions of the ions, electrons, excited atoms and radiation quanta.

For this purpose, gases or gas mixtures are electrically excited by a gas discharge to such an extent that the plasma state just mentioned is achieved. A mixture of neutral gas, electron gas, excited atoms, ions and light quanta is produced. This mixture can subsequently be used for example for surface activation of membranes and other bodies.

The DD 0 272 340 therefore describes for example a flat surfaced coarse pore membrane made of an acrylonitrile polymerisate and a method for its manufacture, in which by means of sequential or simultaneous planiform coating together of a casting solution containing the acrylonitrile polymerisate and a solution incompatible with the latter containing a second polymer and subsequent coagulation, a 2-layer form is shaped, characterised in that before or after the separation of the 2-layer form, the surface layer of the membrane formed from the acrylonitrile polymerisate is subjected to a low temperature plasma treatment in the inert medium with an energy of 330 to 3 300 w.s.cm2 transmitted to the membrane.

The membranes manufactured in this manner are characterised by the pores of approximately uniform size present in the separative layer on the upper side of the membrane. These membranes are suitable for microfiltration or as carriers for the manufacture of composite membranes.

DE-OS 35 09 068 describes pore membranes obtained by the effect of plasma and corona discharge in the presence of substances in gas form on pore membranes and if appropriate, chemical modification. These pore membranes are manufactured in such a way that a pore membrane is subjected to a plasma or corona discharge in the presence of substances in gas form and is subsequently chemically modified if appropriate.

The pore membranes are subsequently used for desalting or increasing the concentration of aqueous or liquid systems that may for example contain colorants.

DE-OS 37 12 491 describes a hydrophobic, microporous microfiltration membrane with a permanently hydrophilic surface and a pore size of approx. 0.1 µm or less for separating particles from aqueous solutions, containing a

hydrophobic, microporous microfiltration substrate with a permanently hydrophilic surface, which has received its permanently hydrophilic surface through treatment with a non-polymerisable plasma gas, with the body of the substrate demonstrating to a great extent the same characteristics such as pore size, hydrophobia, mechanical strength and chemical resistance as the original substrate before the treatment. These membranes are subsequently used for applications in bioreactors.

Membranes treated with low pressure plasma show reduced complement activation (F. Poncin-Epaillard et al., Journal of Applied Polymer Science, Vol. 44, 1513-1522 (1992)), with the plasma gas used for treatment consisting of tetrafluorocarbon or sulphur hexafluoride.

In addition to the fact that extremely dubious substances in the sense of environmental protection are involved here, there is also the disadvantage that the treated membranes show markedly increased thrombogenity. The coating time lasts a few minutes and therefore economically viable manufacture of the modified membrane is not possible.

Membranes that are intended to be suitable for medical purposes must present the highest possible degree of biocompatibility, as was generally described already at the beginning.

Consequently, during dialysis using membranes made of regenerated cellulose, a marked complement activation was observed in addition to other phenomena. The complement system within the blood plasma is a complex plasma enzyme system consisting of many components that in different ways serves for defence against damage by the penetration of foreign cells (bacteria, etc.). If antibodies against the invading organism are present, the complement system can be

activated by the complex of the antibodies with antigenic structures of the foreign cells, otherwise complement activation occurs by an alternative pathway through special surface characteristics of the foreign cells. After activation, these proteins react specifically with another according to a specific sequence and a cytotoxic complex is finally formed that destroys the foreign cell.

Alongside this, peptides such as C5a and C3a are released from individual components, which trigger symptoms of inflammation and which may also occasionally cause unwanted allergic reactions on the body's side. It is assumed that the activation in the case of haemodialysis membranes made of regenerated cellulose occurs via the alternative pathway. The complement activation is objectively observed by determining the complement fragments C3a or C5a.

In this connection, reference is made to the following work: D.E. Chenoweth et al., Kidney International Vol. 24, pages 764 ff, 1983 and D.E. Chenoweth, Asaio-Journal Vol. 7, pages 44 ff, 1984.

Within the context of the present invention, the complement activation is assessed on the basis of the fragment C5a. For this purpose, 250 ml of heparinised blood was recirculated with hollow fibres in vitro over a period of 4 hours with a plasma flow of 100 ml/min. through a dialysis machine with 1 m2 of effective exchange area. The C5a fragments in the plasma were assayed by ELISA (enzyme-linked immunosorbent assay) from the Behring company. The relative complement activation for the respective measuring time was calculated by formation of the ratio from the concentration at the time of taking the sample and the initial value in percent. Reference was made to the measuring value after 4 hours of recirculation time for the assessment. Flat membranes were

incubated with 8 ml of heparinised blood for 3 hours and the C5a concentration was subsequently assayed.

Even though the clinical significance of complement activation has not yet been elucidated, one endeavours to rule the latter out in as far as possible during haemodialysis.

Dialysis membranes may very easily result in haemocoagulation in the artificial kidney during their therapeutic use. This is usually stopped medicinally by administration of heparin. If the dose of heparin is too low however, the thrombogenity of a dialysis membrane may have a detrimental effect on the patient.

Plasmatic coagulation is a complex process governed by enzymes. In a similar manner as with the complement system, approximately 20 plasma proteins combine in their action and to be precise in both promoting and inhibiting, thereby controlling the progress of coagulation.

In principle, plasmatic coagulation may be triggered through two different biological mechanisms. The so-called endogen system plays a major role in this case during activation on foreign surfaced. In addition, injury to the vessel endothelium via the so-called exogenic system has a promoting effect on coagulation. Both routes ultimately result in formation of the central enzyme for coagulation, thrombin. The most important inhibiting substance of thrombin and haemocoagulation overall is antithrombin III.

The inhibition of thrombin by antithrombin III result in TAT (thrombin-antithrombin III complex), to which reference was made within the context of the present invention for assessment of thrombogenity. Since plasma coagulation is promoted by thrombocytes (platelets), the number of

thrombocytes (platelet count) is particularly meaningful as a thrombogenity parameter.

During the dialysis treatment of a renal patient with dialysis machines that contain membranes made of regenerated cellulose, a transient reduction in leukocytes occurs at the outset of dialysis treatment. This effect is known as leukopenia.

Leukopenia is a reduction in the leukocyte count (white blood cells) in the circulation. The WBC count in humans is approx. 4,000 to 12,000 cells/mm3. The leukopenia in dialysis is most pronounced 15 to 20 min. after the beginning of treatment and the neutrophils (which are the leukocytes that may be stained with neutral or simultaneously acid and alkaline colorants) may almost completely disappear. The leukocyte count subsequently recovers within approximately one hour to almost the initial value or exceeds the latter. If a further dialysis machine is connected after leukocyte recovery, leukopenia will recur to the same extent.

Cellulose membranes cause marked leukopenia. Even if the clinical significance of leukopenia has not been scientifically elucidated, a dialysis membrane is desirable for haemodialysis that does not show the leukopenic effect, without detracting as a result from the other very desirable positive properties of dialysis membranes made of regenerated cellulose.

The substances that may likewise influence the biocompatibility of a membrane include albumin and β microglobulin. β microglobulin (molecular weight approx. 11 800) is loosely bound to the surface of all nucleated cells as a part of the main histocompatibility antigen complex. This complex is responsible among other aspects for

the tolerability between foreign tissue and the body's own tissue.

β microglobulin is exclusively broken down in the kidneys and the daily rate of production in healthy individuals is approximately 150 mg. Dialysis patients and uraemic patients have considerably higher plasma β microglobulin concentrations than healthy individuals.

This increase in the β microglobulin concentration in long-term dialysis patients is particularly observed after the use of membranes made of regenerated cellulose and is attributed to the fact that these membranes are less porous in the molecular range of 1,000 to 20,000 and the microglobulin is consequently not removed to a sufficient extent during the dialysis. It is therefore of great interest that the β microglobulin is effectively removed during the treatment.

The albumins likewise belong to the group of serum proteins and represent the largest group therein. Albumins maintain the colloidosmotic pressure and transport endogenous and exogenous low-molecular substances. Furthermore, they constitute the body's protein reservoir. Since the number of albumins is generally reduced in dialysis patients, care should be taken during treatment that the albumin loss remains as limited as possible.

In the case of the different membranes that are suitable for medical purposes and how they have been used for many years for dialysis and ultrafiltration, there is the desire to have types of membrane available that show as little complement activation as possible. At the same time, all the other important parameters characteristic of such a membrane should be maintained, or at least not be impaired.

Up to now however, it has not been possible or only possible using elaborate methods to treat membranes for medical purposes in such a way that a membrane which is able to function properly, but with a low level of complement activation is achieved. Furthermore, the thrombogenity parameters should not be made worse with such a membrane.

The task of the invention was therefore to make a membrane for medical purposes available in which the outer surface can be deliberately influenced in such a way that its biocompatibility, particularly with regard to C5a complement activation, is increased.

This problem is solved in the case of a membrane according to the generic term of claim 1 in that the reduction in C5a complement activation is at least 50% and the reduction in thrombogenity is at least 5%.

Preferably, the degree of reduction in C5a complement activation is even greater and amounts to more than 85% and in particular more than 90%.

The membrane according to the invention is furthermore characterised in that it demonstrates improved thrombogenity in relation to the unmodified membrane.

Preferably, its essential transport and separating properties, at least with reference to the ultrafiltration rate, the dialysis performance for vitamin B12 and creatinine and the filtering coefficients for albumin and β microglobulin are identical or improved in relation to the unmodified membrane, or at least not considerably worsened. Not considerably worsened means at best in the order of 10%, preferably however less than 10%.

Synthetic or natural polymers or mixtures thereof are used as the membrane material.

In the development of the invention, polyacrylonitrile, polysulphone, polyethersulphone, sulphonated polyethersulphone, polyvinylidene fluoride, polycarbonate, polypropylene, nylon, polystyrene and/or polyurethane were used as synthetic polymers.

In a further advantageous development of the invention, rengenerated cellulose and cellulose derivatives were used as natural polymers.

The membrane according to the invention may be finished in different forms and therefore for example as a hose membrane, a hollow fibre membrane or as a flat membrane.

In one version of the invention, both surfaces of the membrane are modified by treatment with a low-pressure plasma.

The problem is likewise solved by a method for manufacturing a membrane for medical purposes by treatment with a low-pressure plasma, characterised by passing an untreated dialysis membrane through a chamber in which low-pressure plasma is present at a rate of more than 2 m/min with reference to a plasma treatment line of 10 to 30 cm.

Preferably, the rate is more than 50 m/min.

It is also possible not to conduct the method continuously, with cut off membrane films (in the case of flat membranes) or generally membrane part sections are treated individually with a low-pressure plasma. On the other hand, transport rates of 2 to 200 m/min are possible for the membrane with continuous methods.

The non-continuous operating method may be conducted in such a way that a continuous belt is passed section by section through the plasma treatment line and is moved forward after

the corresponding treatment time. Preferably however, the non-continuous treatment method is conducted with specific membrane components. The treatment can be performed on either a single side or both sides.

It is appropriate that the energy density of the plasma produced during the treatment should be approx.

$$0.00 \text{ to } 1.2 \frac{ws}{cm2} .$$

The duration of treatment and length of stay in the plasma line is generally between 0.1 and 10 seconds.

By means of the treatment method according to the invention, the surface of the membrane is beneficially modified. The modifications in the surface generally cover an area of approx. 1 to 2 nm.

It goes without saying that with the existence of plasma treatment lines of other than 10 to 30 cm, the rate is adapted to the corresponding dimensions.

Treatment is preferably performed with sulphur dioxide, water, air, oxygen, nitrogen, a mixture of methane and oxygen, alone or in a mixture with inert gases, preferably argon.

The invention is explained by the examples below:

Example 1

A Cuprophan® size 12 x 32 cm flat membrane was plasma treated in a vacuum chamber. For this purpose, the flat membrane was tightened on the inside of a copper cylinder, which was subsequently mounted on a test plate in the vacuum chamber. The copper cylinder and the chamber wall formed the electrodes for the high frequency (13.56 MHz); an intensive

and uniform plasma burned inside the copper cylinder. The flat membrane was therefore treated on one side only.

Treatment parameters:

Gas mixture: SO2/argon (30%)

Overall gas glow: 150 ml/min.

Overall pressure: 1.8×10^{-3} mbar

The duration of treatment was 24 seconds.

The plasma treated flat membrane showed a reduction in the C5a complement activation of 88% in comparison with the untreated flat membrane.

The values for the ultrafiltration rate, the dialysis performance for NaCl, vitamin B12 and urea were respectively unmodified within the context of the usual measurement accuracy before and after the plasma treatment. The values for the force at rupture and elongation at rupture of the membrane also remained unmodified.

Example 2

According to example 1, an SPES flat membrane (Vitrex 5200 G) instead of the Cuprophan flat membrane was treated for 28 seconds with the gas mixture of example 1. All the other experimental conditions corresponded to those of example 1.

The plasma treated SPES membrane showed a reduction in C5a complement activation of 86%.

The remaining performance data of the membrane did not significantly change.

Example 3

A Cuprophan® flat membrane 25 cm wide wound on to a roll was drawn at a rate of 2 m/min. through a plasma treatment chamber of 50 x 30 cm in size. The plasma (13.56 MHz HF) was injected via a hollow cathode, through which spatial concentration was achieved. A water/argon mixture (30% argon) was used as the plasma gas.

The reduction in the C5a complement activation was 77.2% in comparison to the untreated membrane, whilst the remaining data did not show any significant changes.

Example 4

The same procedure was followed as in example 3, with the film rate being 25 m/min.

The reduction in the C5a complement activation was 91.2%, whilst the remaining data did not show any significant changes.

CLAIMS

1. A dialysis membrane for medical purposes modified with a low-pressure plasma, which consequently shows a reduction in complement activation and thrombogenity in comparison with the unmodified dialysis membrane, characterised in that the reduction in complement activation is at least 50% and the reduction in thrombogenity is at least 5%.

2. A membrane according to claim 1, characterised in that the reduction in complement activation is more than 85%

3. A membrane according to claim 1, characterised in that the reduction in complement activation is more than 90%.

4. A membrane according to one or more of claims 1 to 3, characterised in that their essential transport and separating properties, at least with reference to the ultrafiltration rate, the dialysis performance for vitamin B12 and creatinine and the filtering coefficients for albumin and β microglobulin are identical or improved, or at least not considerably worsened in relation to the unmodified membrane.

5. A membrane according to one or several of claims 1 to 4, characterised in that synthetic or natural polymers or mixtures thereof are used as the membrane material.

6. A membrane according to claim 5, characterised in that polyacrylonitrile, polysulphone, polyethersulphone, sulphonated polyethersulphone, polyvinylidene fluoride, polycarbonate, polypropylene, nylon, polystyrene and/or polyurethane are used as synthetic polymers.

7. A membrane according to claim 5, characterised in that regenerated cellulose and cellulose derivatives are used as natural polymers.

8. A membrane according to one or several of claims 1 to 7, characterised in that a hose membrane, hollow fibre membrane or a flat membrane is involved.

9. A membrane according to one or several of claims 1 to 8, characterised in that one surface only of the membrane is modified by treatment with a low-pressure plasma.

10. A membrane according to claim 9, characterised in that the surface involved in this case is the surface that will subsequently face the blood during a dialysis.

11. A membrane according to one or several of claims 1 to 8, characterised in that both surfaces of the membrane are modified by treatment with low-pressure plasma.

12. A method for manufacturing a membrane for medical purposes by treatment with low-pressure plasma according to claim 1, characterised by passing an untreated dialysis membrane through a chamber in which low-pressure plasma is present at a rate of more than 2 m/min with reference to a plasma treatment line of 10 to 30 cm.

13. A method according to claim 12, characterised in that the rate is more than 50 m/min.

14. A method according to one or several of claims 12 to 13, characterised in that the treatment is performed with sulphur dioxide, water, air, oxygen, nitrogen, a mixture of methane and oxygen, alone or in a mixture with inert gases, preferably argon.

15. A method for manufacturing a membrane for medical purposes by treatment with a low-pressure plasma according to claim 1, characterised in that the membrane is treated for a period of 0.1 to 10 seconds with a plasma energy density of 0.04 to 1.2 Ws/cm2.

16. A method according to claim 12, characterised in that the membrane is treated for a period of 0.1 to 10 seconds with a plasma energy density of 0.04 to 1.2 Ws/cm2.